

Detection of thyroid disrupting chemicals using in vitro and ex vivo assays

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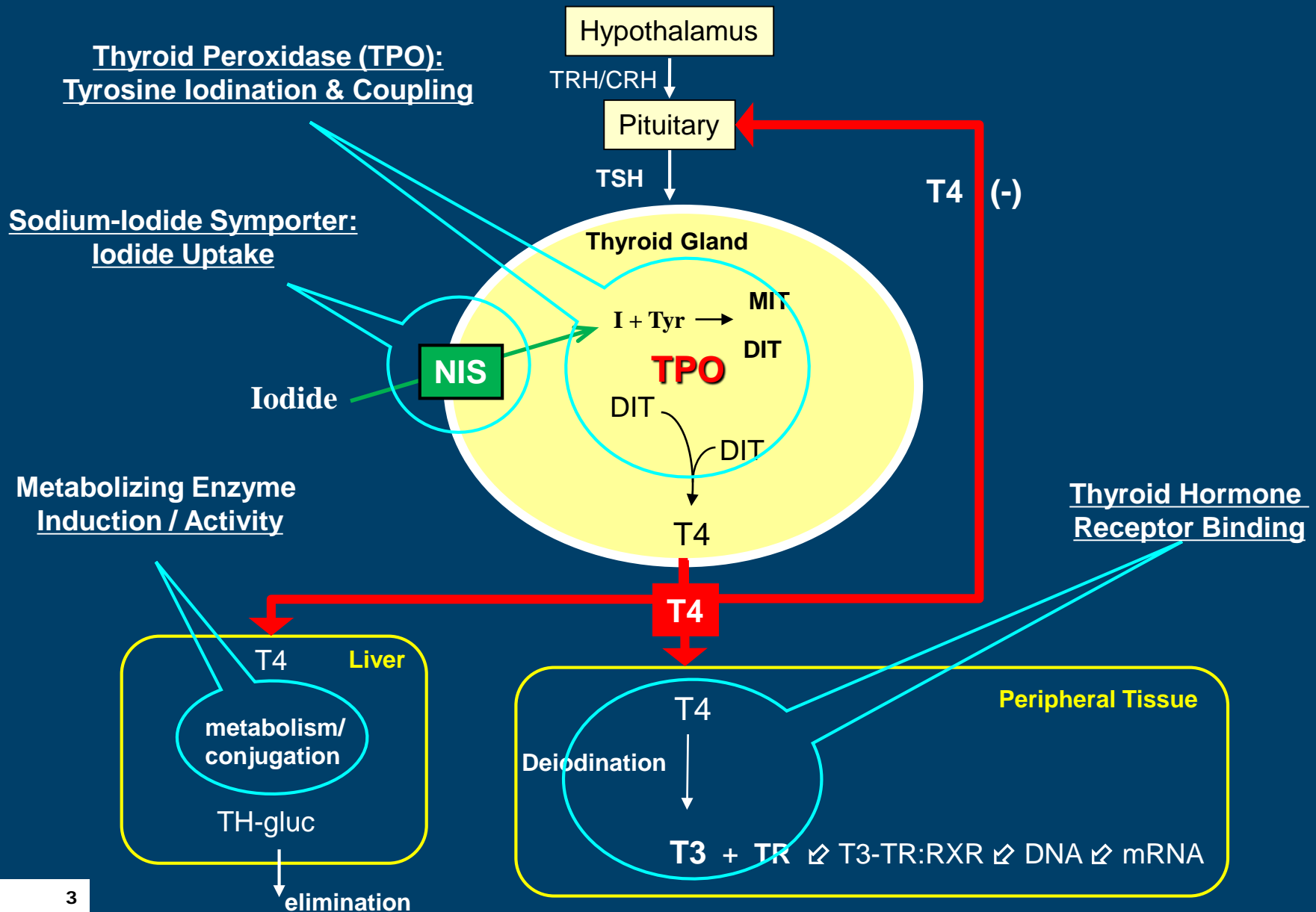
Background

- SDWA/FQPA requires EPA assess chemicals for endocrine activity
 - Many chemicals with limited or no information on thyroid activity
- For registration of new chemicals or re-evaluation/registration of existing chemicals, full in vivo tests would be prohibitively expensive and time consuming
- There is a push in toxicology to use less in vivo and more in vitro based methods for prioritizing chemicals for screening and evaluation

Objectives

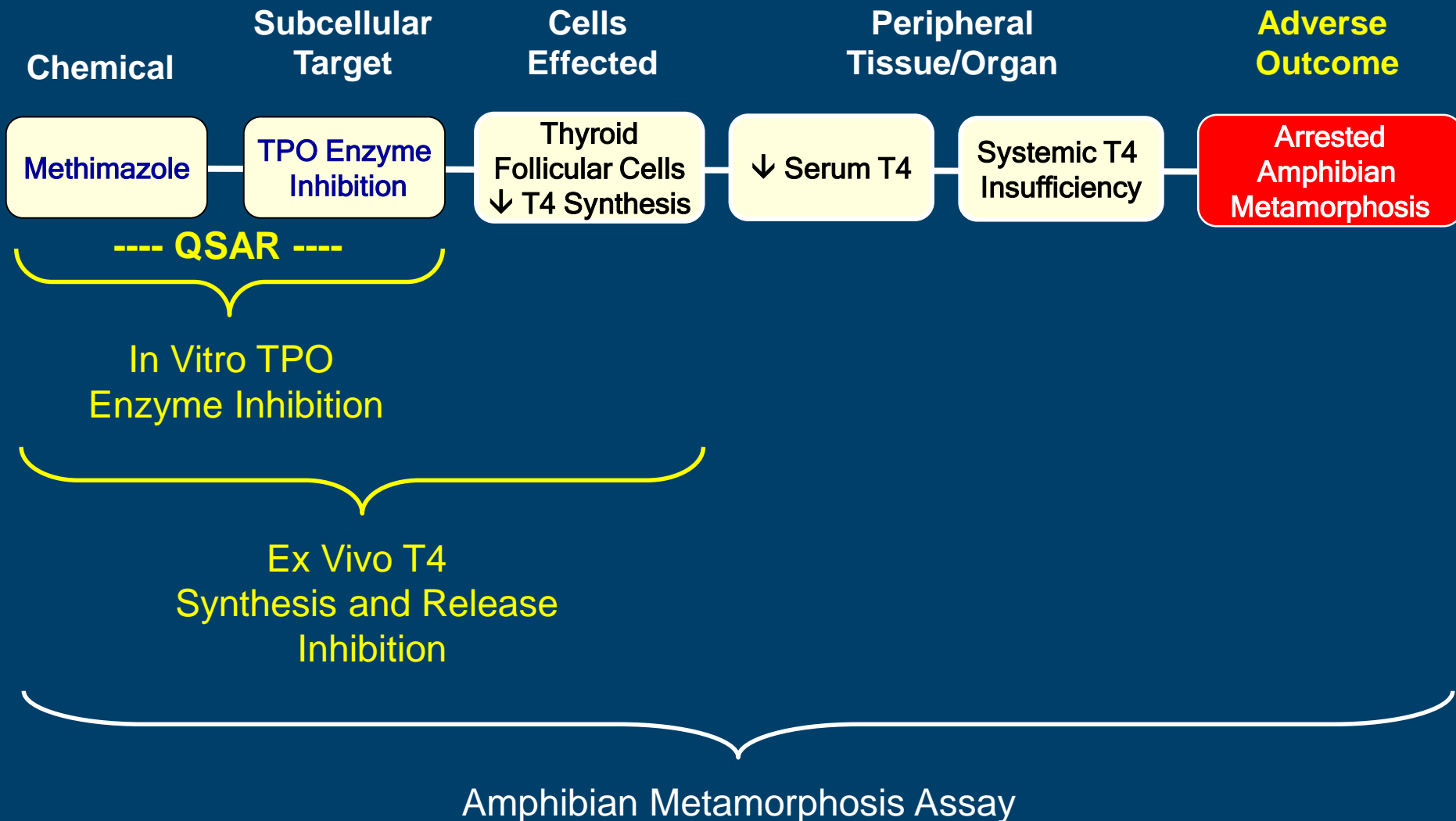
- Develop higher throughput, mechanism-based, predictive tools
- Support the development of structure activity relationship models for thyroid hormone disrupting activity

Chemical Interaction with the HPT Axis

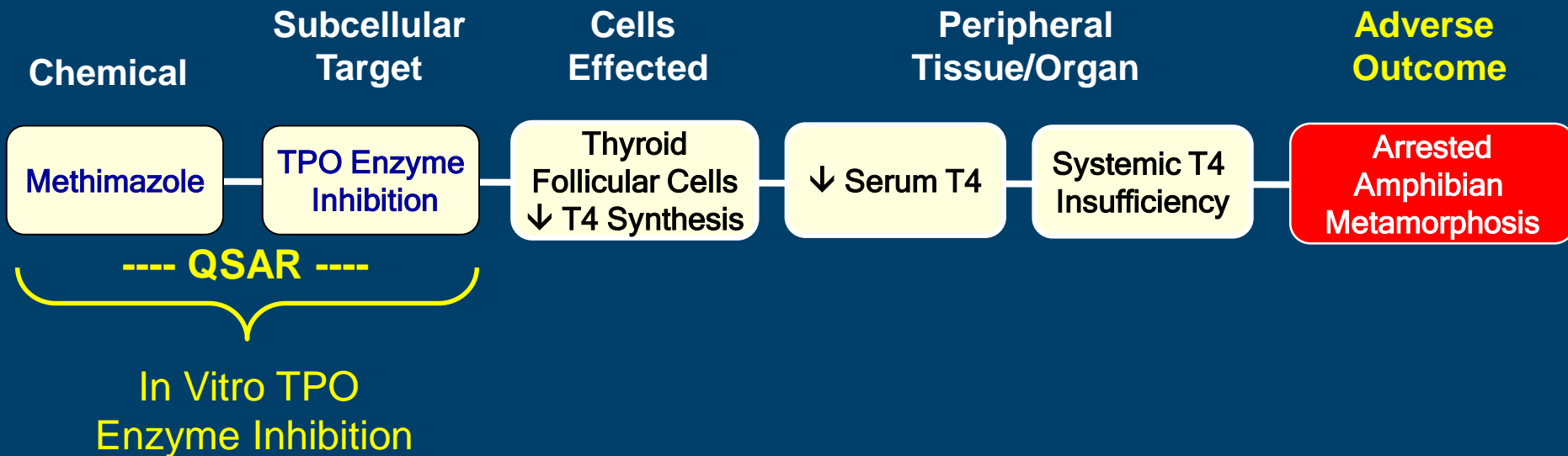


Approach:

Tests Across Multiple Levels of Biological Organization



Assay at the initial chemical – biological interaction

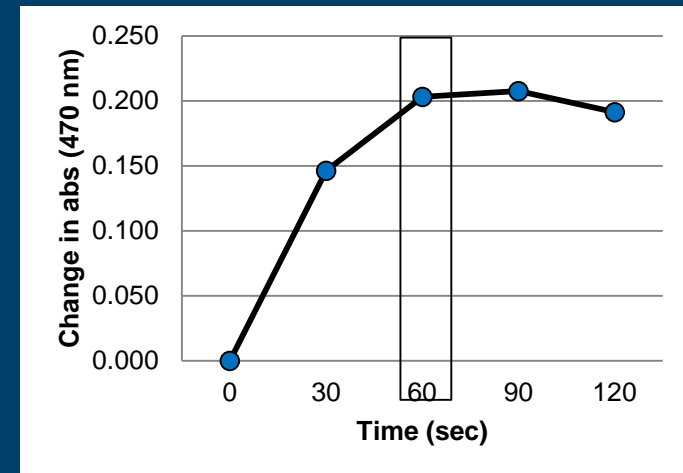
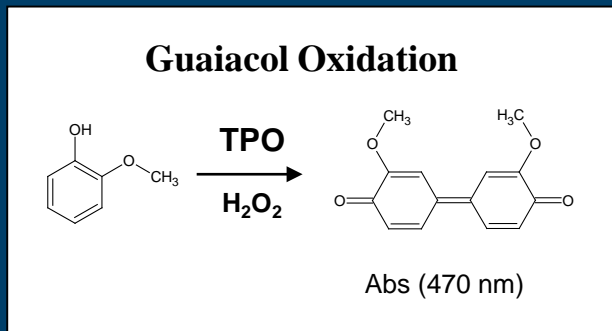
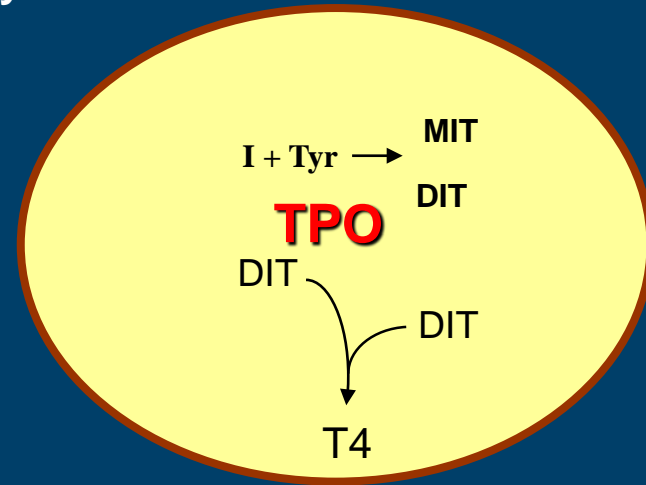


TPO Inhibition Assay

T4 Synthesis Inhibition

- In Vitro Assay
- Thyroid Microsomes (porcine)
- Thyroid Peroxidase Inhibition

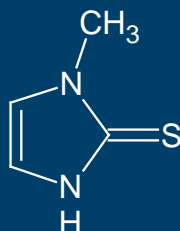
Thyroid Gland



Developing the In Vitro Assays

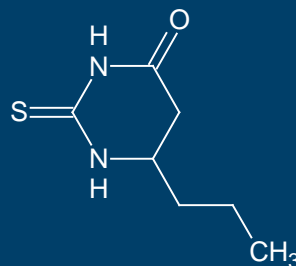
➤ Select model T4 synthesis inhibitors

➤ methimazole

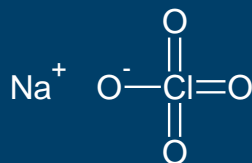


TPO Inhibitors

➤ propylthiouracil (PTU)

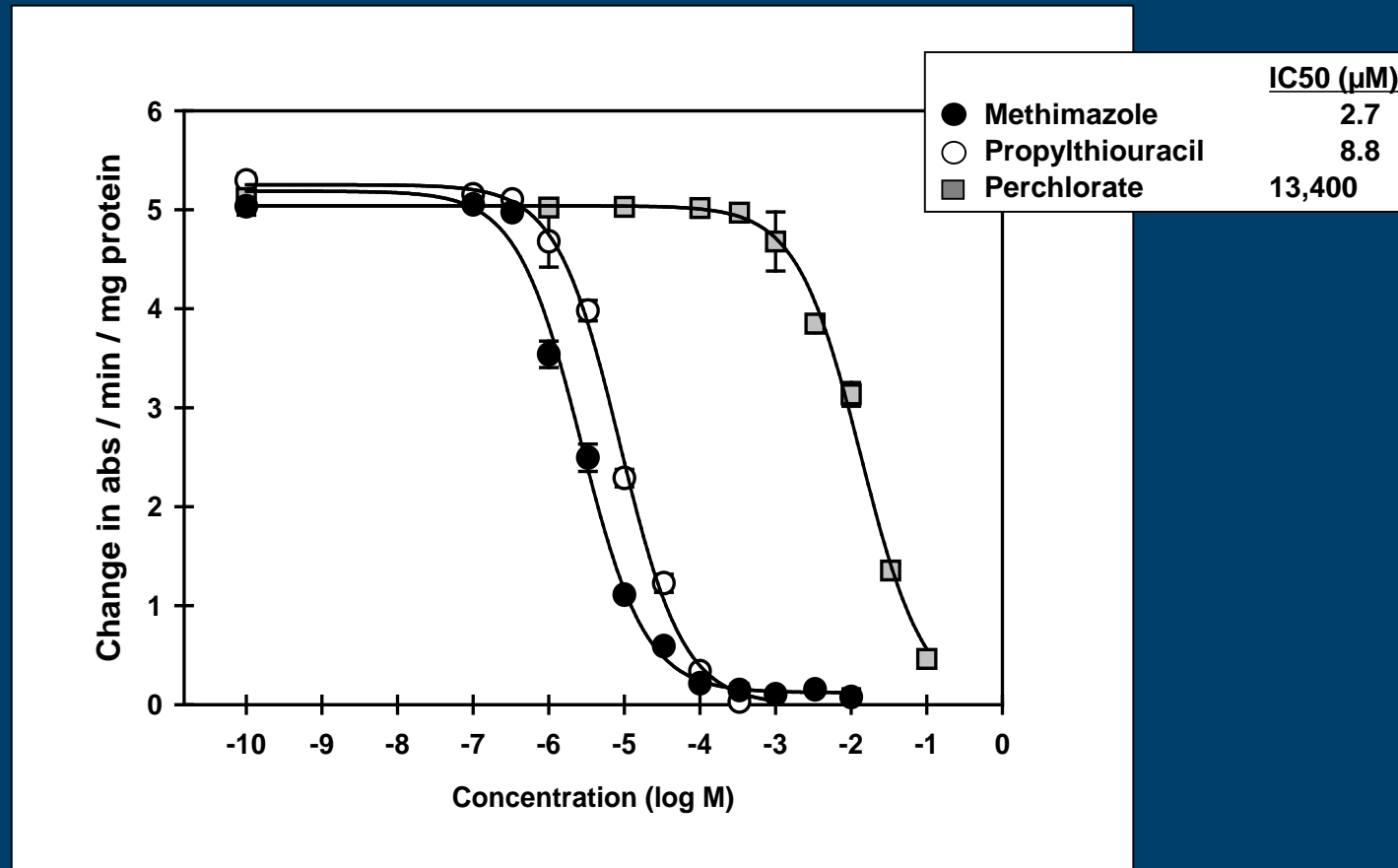


➤ perchlorate



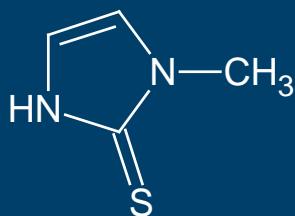
NIS Inhibitor

In Vitro TPO Inhibition by Model T4 Synthesis Inhibitors

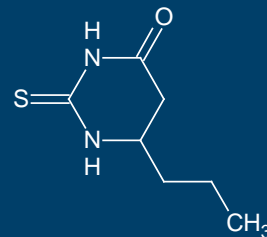


Selection of Chemicals to Test in the In Vitro Assays

- Test additional chemicals based upon structural similarity to known active chemicals

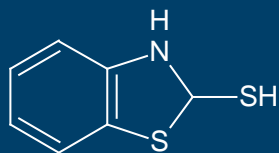


methimazole

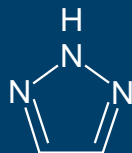
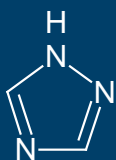


PTU

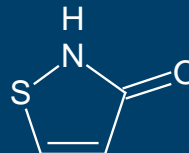
Test chemical classes



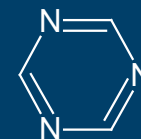
thiazoles and
benzothiazoles



triazoles



isothiazolinones

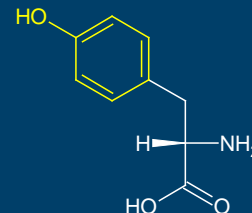


triazines

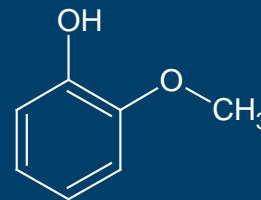
Selection of Chemicals to Test in the In Vitro Assays

➤ Test additional chemicals based upon structural similarity to known TPO substrates: potential competitive inhibitors

- Endogenous substrate: tyrosine

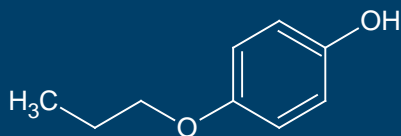


- TPO enzyme assay substrate: guaiacol (o-methoxyphenol)

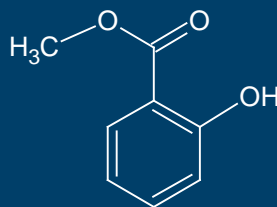


Test Chemical Classes

alkoxyphenols



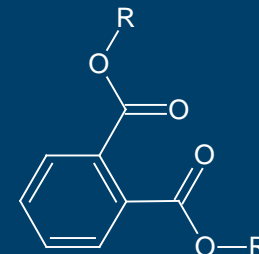
salicylates



benzoates

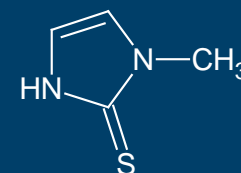
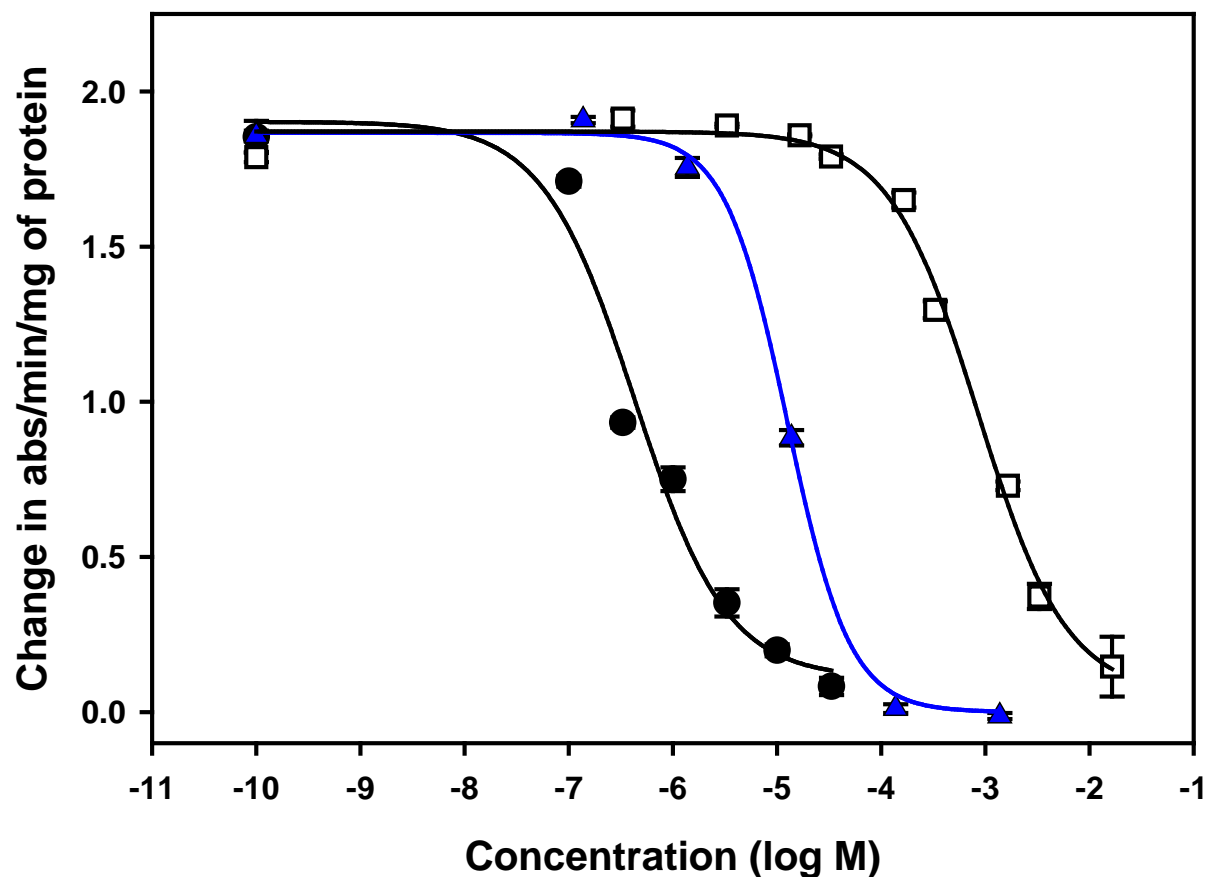


phthalates

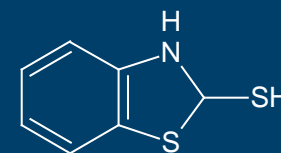


Identification of TPO inhibitors

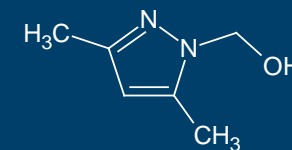
TPO Inhibition by mercaptobenzothiazole (MBT) and dimethyl hydroxymethylpyrazole (DMP)



● Methimazole



▲ MBT



□ DMP

TPO Inhibition Assay Summary

Structural Similarity to Methimazole

Chemical Name	TPO Inhibition	TPO Inhibitory Potency Relative to Methimazole
Methimazole	(+)	1
2-Mercaptobenzothiazole (MBT)	(+)	0.042
1,2-Benzisothiazoline-3-one	(-)	
Dimethyl hydroxymethyl pyrazole	(+)	0.00071
Drometrizole	(-)	

Structural Similarity to PTU

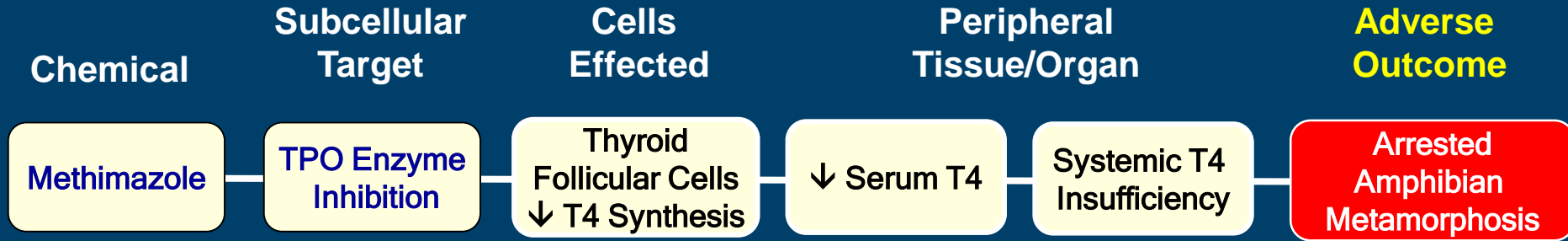
Chemical Name	TPO Inhibition	TPO Inhibitory Potency Relative to Methimazole
Propylthiouracil (PTU)	(+)	0.17
Trichloromelamine	(-)	
Terbuthylazine	(-)	

TPO Inhibition Assay Summary

Structural Similarity to TPO Substrates: Phenolics, etc.

Chemical Name	TPO Inhibition	TPO Inhibitory Potency Relative to Methimazole
Nonylphenol; n-chain	(-)	
Nonylphenol; branched	(-)	
n-Octylphenol	(-)	
n-Butylphenol	(-)	
n-Propylphenol	(-)	
n-Ethylphenol	(-)	
n-Methylphenol	(-)	
Phenol	(-)	
4-Propoxyphenol	(+)	~ 0.0003
Methylsalicylate	(-)	
Butyl salicylate	(-)	
Methyl, 2-methylbenzoate	(-)	
Diethylphthalate	(-)	
Benzylbutylphthalate	(-)	
Triclosan	(-)	

Test at Next Higher Level of Biological Organization



MMZ

MBT

DPM

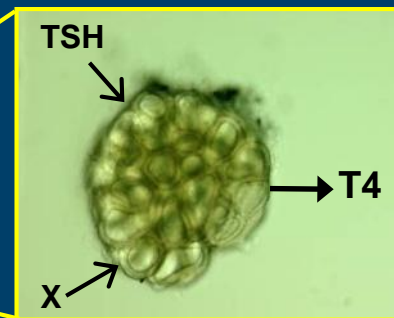
POP

→ + → ?

Ex Vivo T4
Synthesis and Release
Inhibition

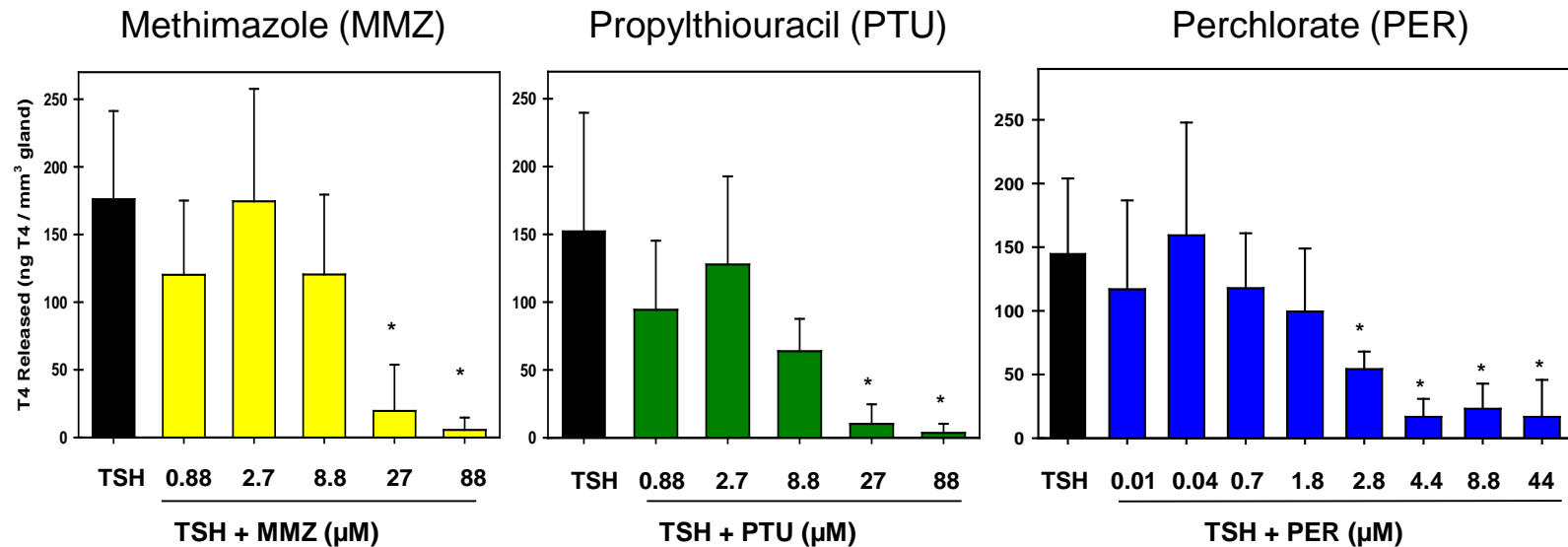
In Vitro Assays: Thyroid Explant Cultures

- *X. laevis* explant culture assays
 - Dissect glands from *X. laevis* tadpoles
 - Culture in 96-well plates
 - Inhibition of bTSH stimulated T4 synthesis and release



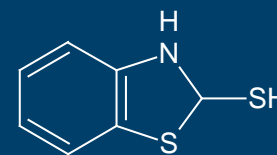
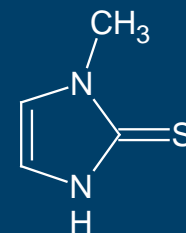
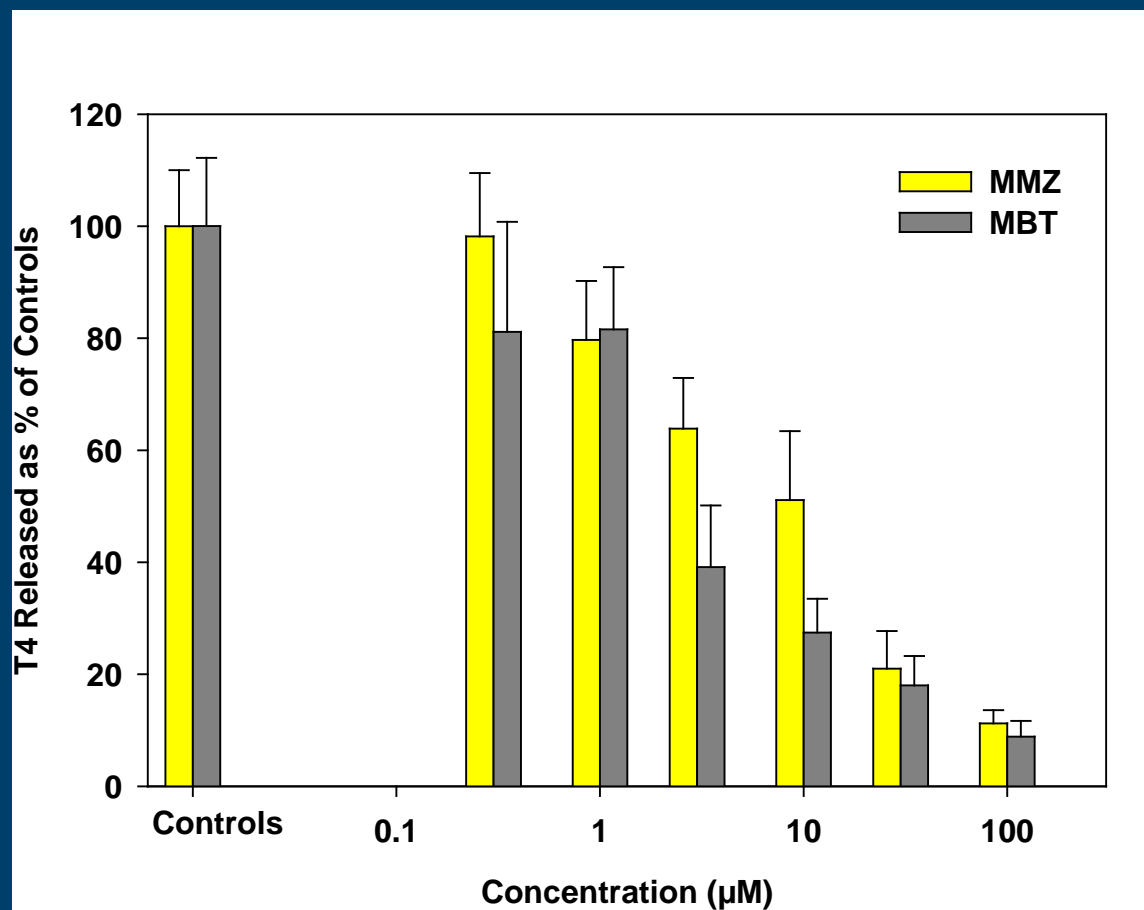
In Vitro Assays: Thyroid Explant Cultures

1. Confirm Inhibition of T4 Release by Model TH Synthesis Inhibitors



In Vitro Assays: Thyroid Explant Cultures

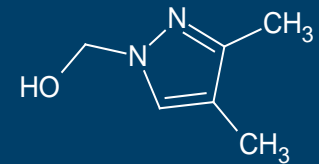
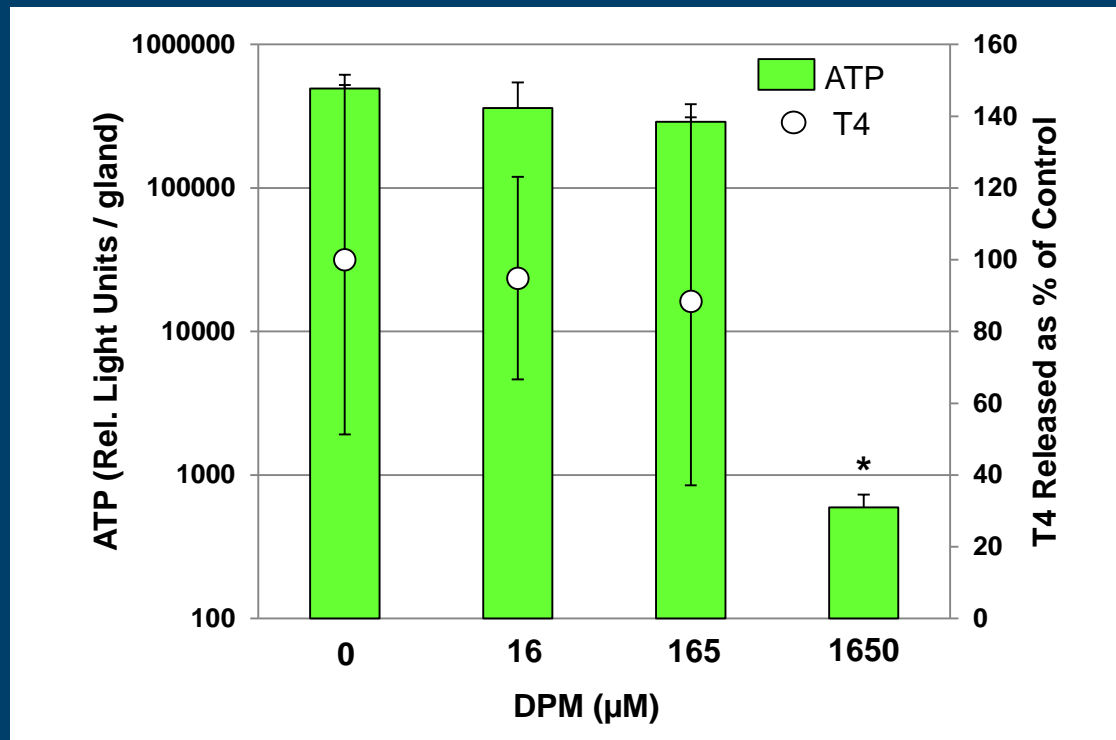
2. Test positives from TPO inhibition assay



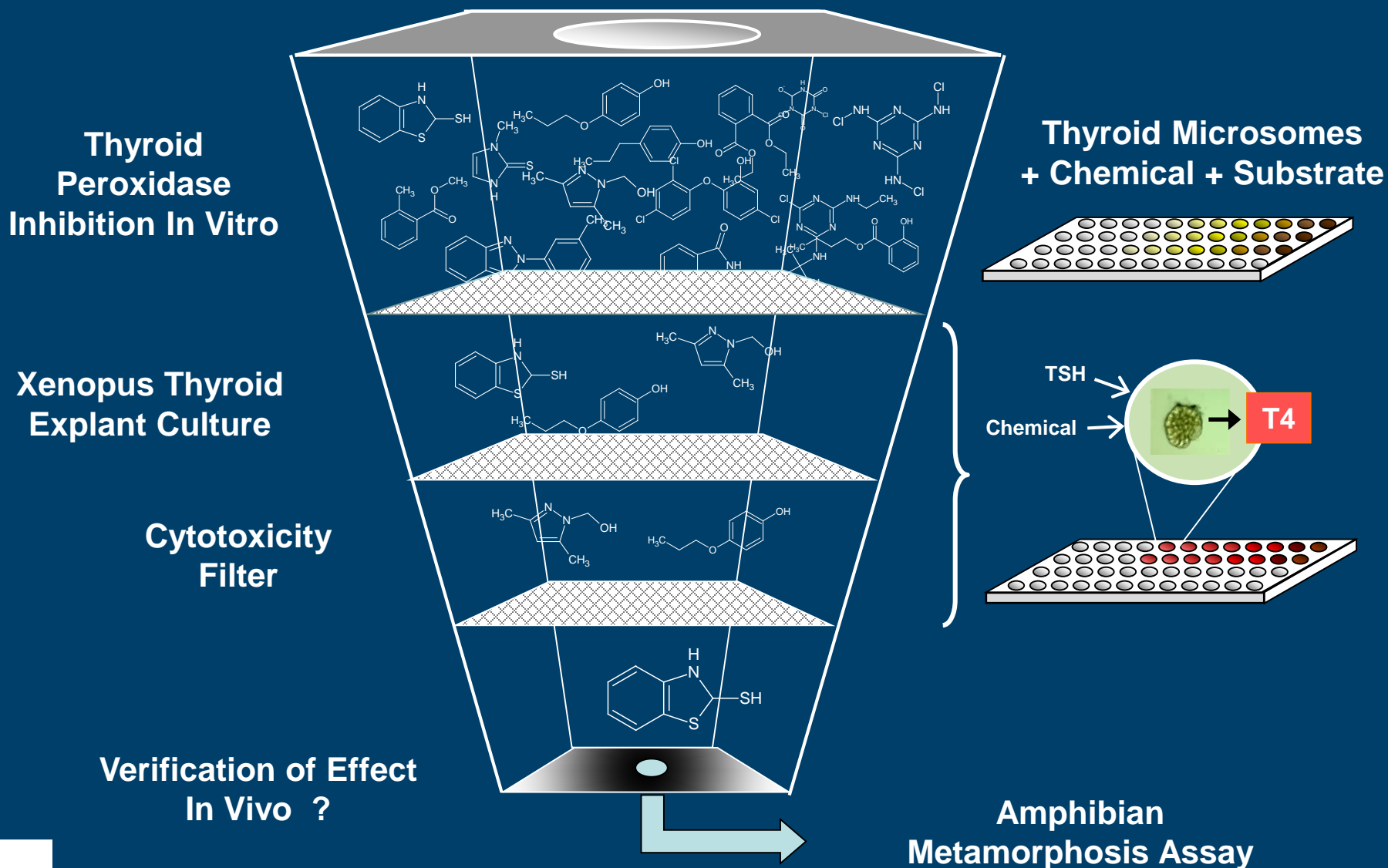
In Vitro Assays: Thyroid Explant Cultures

- TPO inhibitors may be toxic to the cultured glands

Gland Viability (ATP)



Testing Across Multiple Steps in a Pathway for Prioritization of Chemicals for Further Testing



Acknowledgements

Sig Degitz

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